WHITE SEA HERRING (*CLUPEA PALLASI MARISALBI*) POPULATION STRUCTURE: INTERPOPULATION VARIATION IN FREQUENCY OF CHROMOSOMAL REARRANGEMENT

by

Dmitry L. LAJUS (1)

ABSTRACT. - Chromosomal sets of the White Sea herring (*Clupea pallasi marisalbi*, Berg) were studied to resolve the problem of its population structure. Adults, larvae and embryos were analyzed. It was found that populations from different locations differ in frequency of Robertsonian rearrangement ($2n = 52, 51, 50$; $NF = 60$). Frequency of rearrangement increases from north-west (0.000-0.005) to south-east (0.133) of the White Sea. Chromosomal differences between fast- and slow-growing herring have not been found. According to karyological data, the White Sea herring subspecies can be considered as a group of populations of Pacific origin with only a poor gene flow between them.

RÉSUMÉ. - Structure de la population de hareng (*Clupea pallasi marisalbi*) de la Mer Blanche: variabilité entre populations dans la fréquence de réarrangement chromosomique.

Les caryotypes de hareng (*Clupea pallasi marisalbi*, Berg) de la Mer Blanche ont été etudiés pour définir la structure de la population. Des embryons, des larves et des individus adultes ont été analysés. La seule différence entre les populations provenant de diverses localités est celle de la fréquence de réarrangement chromosomique de Robertson. La fréquence de ce réarrangement chromosomique augmente du nord-ouest (0,000-0,005) au sud-est (0,133) de la Mer Blanche. Aucune différence chromosomique n'a été détectée entre les individus à croissance lente ou rapide. Selon les données caryologiques, on peut considérer les sous-espèces de hareng de la Mer Blanche comme un groupe de populations originaires du Pacifique, avec un faible échange de gènes.

Key-words. - Clupeidae, *Clupea pallasi marisalbi*, PNE, White sea, Chromosomal polymorphism, Population structure.

Herring, having a large significance for fisheries, have been the object of intensive population studies. However, despite a considerable body of data available, important questions of population structure remain unresolved. A prime example of this is the White Sea herring (*Clupea pallasi marisalbi* Berg), a subspecies of Pacific herring (Rass, 1985; Rass and Wheeler, 1991; Whitehead, 1985). The position of the White Sea herring in the genus *Clupea* and the interrelations between its stocks are yet unclear.

Most researchers believe that stocks of the White Sea herring are genetically isolated. Some authors (Dushkina, 1975, 1988; Skvortzova, 1975) assume that these stocks differ in origin and "...have a systematic range not lower, than that of subspecies, or even species" (Dushkina, 1975, p. 249). The opponents consider that the White Sea herring is a single population, "a single biological whole" with diversity a consequence of high

---

(1) Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, 199034, St.-Petersburg, RUSSIA.

Cybium 1996, 20(3): 279-294.

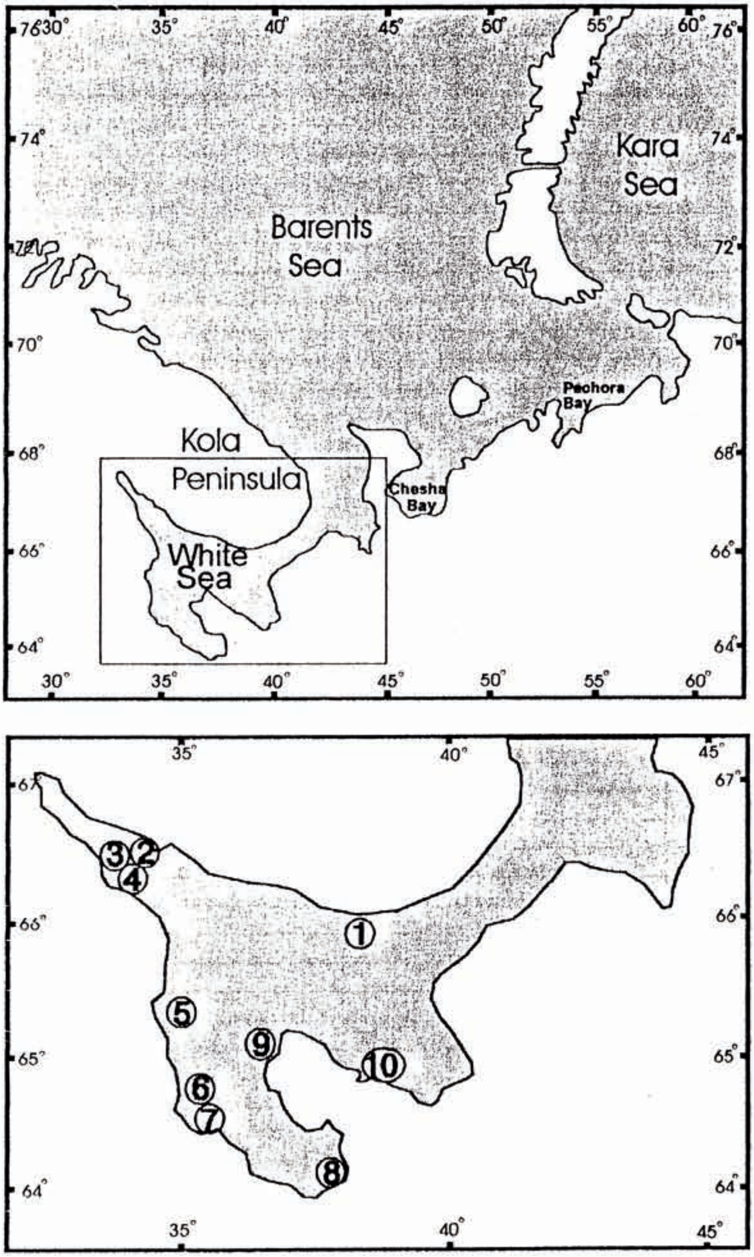

Fig. 1. - Sampling locations of herring in the White Sea: 1. Chavanga/Basin-Tersky coast (66°03'N, 37°46'E); 2. Pila/Kandalaksha Bay (66°44'N, 34°10'E); 3. Kasian/Kandalaksha Bay (66°34'N, 33°28'E); 4. Chupa/Kandalaksha Bay (66°28'N, 33°30'E); 5. Pongoma/Basin-Karelsky coast (65°22'N, 34°28'E); 6. Sorokskaya/Onega Bay (64°32'N, 34°49'E); 7. Kolezhma/Onega Bay (64°25'N, 36°00'E); 8. Kiy/Onega Bay (64°15'N, 37°55'E); 9. Pushlakhta/Basin-Liamitzky coast (64°49'N, 36°34'E); 10. Suzma/Dvina Bay (64°45'N, 36°02'E).

ability to vary the mode of life and the growth rate (Lapin, 1966; Lapin and Pokhiliuk, 1993).

Karyological studies often yield important information on fish population structure and evolution (Vasil'ev, 1985). Previous studies of the White Sea herring (Skvortzova, 1974a, 1975; Krysanov, 1978) indicated considerable chromosomal variation between stocks. The aim of this paper is to use karyological analysis to resolve problems of the White Sea herring population structure.

MATERIAL AND METHODS

Data were collected in 1985-1990. Sampling covered most of the area of the White Sea herring (Fig. 1). Adults, larvae and embryos were used for chromosomal analysis.

Adults

Adult fishes were caught with gillnets, seines and bottom trawls. They were measured from the tip of the head to the end of the middle rays of the caudal fin. Age was estimated by scale reading and analysis of otholiths. Fishes were categorized as slow- or fast-growing according to their age and size (Rabinerson, 1925; Averintsev, 1927, 1928; Lajus, 1989b). The average length of fast-growing herring is 4-8 cm more than that of slow-growing individuals (Dmitriev, 1946).

The number of vertebrae in juvenile fishes (23 specimens from Sorokskaya Inlet, August 1990) was counted in order to differentiate the White Sea herring from the Atlantic stock. Fishes with 51-55 vertebrae were classified as White Sea herring (7 specimens), while Atlantic herring had 56-59 vertebrae (16 specimens, their karyotype is described in Lajus (1994)).

Fishes were kept in fish-wells (1 x 1 x 1 m) in the sea or in tanks of 100-400 l.

The attempts to apply the most-used fish chromosome techniques (McPhail and Jones, 1966; Denton, 1973) for spawning herring were not successful due to a low number of suitable mitotic plates. Therefore the technique of Gold (1974), with modifications by Shelenkova (1986), and with some adaptations for the herring described below, was applied.

An intramuscular injection of colchicine (0.1-0.5%) was used. Dose and exposition of colchicine depend considerably on the temperature (studies were carried out at the temperature range from -1° up to +18°C). At low temperature (-1° to +5°C) the best results were obtained at the colchicine dose 0.02-0.04 mg per g of fish weight and at an exposition of 8-12 h. At high temperatures (+10° to +15°C) the optimal colchicine dose was 0.003-0.005 mg/g, exposition 2.5-4 h. Cleaned pieces of kidney were suspended in hypotonic solution (0.55% KCl) at room temperature. After 12-13 min hypotonic processing was stopped by adding 1-2 drops of fixative (ethanol-acetic acid 3:1) and then the samples were centrifuged at 1000-1200 rev/min for 5-6 min. Fixative was replaced at least twice prior to preparing slides by air-drying. Slides were stained in 3-5% Giemsa solution (pH = 6.8) for 10-12 min.

Three or more metaphase plates were analyzed from each fish (Table I).

Larvae

Larvae were obtained from eggs collected at the spawning grounds at the end of the incubation period (slow-growing herring), or from artificially fertilized eggs incubated in the laboratory (fast-growing herring) (Table I). Newly hatched larvae (1-2 days after hatching) were analyzed by the method of colchicine baths (Skvortzova, 1975). Larvae were kept in colchicine solution (0.005-0.01%) at a temperature near 10°C for 3-8 h. To

Table I. - Results of the White Sea herring karyotyping. 1)A - adult, L - larvae, E - embryos on the stage of early eye-pigmentation; 2)for larvae and embryos the number of samples is given in brackets; 3)embryos were collected in natural spawning grounds; 4)larvae were obtained after incubation in laboratory of embryos, collected in natural spawning grounds; 5)larvae were obtained in laboratory after artificial fertilization and incubation of embryos.

Location	Data of sampling	Growth rate	Matherial used 1)	Number of specimens observed 2)	Number of metaphase plates observed	Number of specimens with 2n =		
						52	51	50
Chavanga	Aug 1986	Slow	A, feeding	29	111	29	-	-
Chavanga	Aug 1990	Slow	A, feeding	32	145	32	-	-
Pila	Aug 1990	Slow	A, feeding	29	155	29	-	-
Chupa	Apr 1985	Slow	A, spawning	21	92	21	-	-
Chupa	May 1985	Slow	A, spawning	5	32	5	-	-
Chupa	May 1985	Fast	A, spawning	7	30	7	-	-
Chupa	Jun 1985	Slow	A, spawning	3	14	3	-	-
Chupa	Jun 1985	Fast	A, spawning	8	32	8	-	-
Chupa	Jun-Aug 1985	Slow	A, feeding	18	93	18	-	-
Chupa	Apr 1986	Slow	A, spawning	43	140	43	-	-
Chupa	May 1986	Slow	E3)	248(3)	248	246	2	-
Chupa	May 1986	Fast	A, spawning	2	12	2	-	-
Chupa	Jun 1986	Slow	A, spawning	5	21	5	-	-
Chupa	Jun 1986	Slow	L4)	18(2)	18	18	-	-
Chupa	Jun 1986	Fast	A, spawning	31	112	30	1	-
Chupa	Apr 1987	Slow	A, spawning	32	112	32	1	-
Chupa	Apr 1987	Fast	A, spawning	7	30	7	-	-
Chupa	May 1987	Slow	E3)	511(5)	511	509	2	-
Chupa	Jun 1987	Slow	L4)	44(3)	44	44	-	-
Chupa	Jun 1987	Fast	A, spawning	29	124	29	-	-
Chupa	Jun 1987	Fast	L5)	81(5)	81	81	-	-
Chupa	Jun-Aug 1987	Slow	A, feeding	17	92	17	-	-
Kasian	May 1988	Slow	E3)	321(3)	321	320	1	-
Pongoma	Aug 1986	Slow	A, feeding	8	30	8	-	-
Pongoma	Aug 1990	Slow	A, feeding	24	93	24	-	-
Sorokskaya	May 1986	Slow	A, prespawning	16	59	12	4	-
Sorokskaya	May 1986	Fast	A, prespawning	14	48	12	2	-
Sorokskaya	Aug 1990	Slow	A, feeding	36	110	32	4	-
Kolezhma	May-Jun 1986	Slow	A, spawning	20	73	19	1	-
Kolezhma	May-Jun 1986	Fast	A, spawning	8	35	8	-	-
Kolezhma	May-Jun 1987	Slow	A, spawning	73	258	67	6	-
Kolezhma	May-Jun 1987	Fast	A, spawning	12	40	10	2	-
Kolezhma	Jun 1987	Slow, fast	E3)	713(7)	713	649	61	1-
Kiy	May-Jun 1988	Slow	A, spawning	35	114	31	4	-
Kiy	Aug 1990	Slow	A, feeding	36	119	30	5	1
Pushlakhta	Aug 1986	Slow	A, feeding	63	199	50	10	3
Pushlakhta	Aug 1990	Slow	A, feeding	34	101	30	4	-
Suzma	Aug 1990	Slow	A, feeding	45	183	34	10	1

prepare cell suspensions, 50-100 larvae per sample were used. Further processing was the same as for adults.

Embryos at the stage of early eye-pigmentation

Eggs (1000-2000) were collected from littoral or sublittoral vegetation (*Fucus, Ascophyllum*) on sites of the spawning ground where egg mortality was not observed.

Capsules of eggs were separated by passing through suitable gauze in petri dishes with physiological solution at room temperature. Colchicine was added to the solution to make the concentration about 1 mkg/ml for 1 h. After this, physiological solution was carefully replaced with hypotonic solution (0.55 KCl) for 15 min (temperature 20-25°C), and fixed for 2-12 h. Then pieces of embryos were suspended and air-dried slides were made. The quality and quantity of metaphase plates were greatly dependent on the stage of embryogenesis. The best stage for analysis was at beginning of the eye-pigmentation. Under optimal conditions it is possible to obtain 200-300 well-spread metaphase plates per slide; 40-150 plates in each slide were usually analyzed (Table I).

Blastula embryos

A modification of the squash technique (Prokofieva, 1935; Svardson, 1945; Skvortzova, 1975) was used.

Embryos were obtained by means of artificial fertilization. The karyotypes of parents, and embryos on the stage of early eye-pigmentation from the same samples, were analyzed subsequently. The mortality of eggs during the incubation period was not observed. 3-7 consequent fixations in ethanol-acetic fixative (3:1) were made. At the temperature of incubation, -1°+1°C, first fixations were made after 3-4 days from the beginning of incubation (intervals 12-24 h). The higher the temperature the less was the period to the first fixation and the intervals between subsequent fixations. The capsule of the egg was destroyed to separate an embryo. Then the embryo was squashed between two coverglasses, dried, and hydrolyzed in 6N HCl for 5-10 min to avoid overstaining the cytoplasm. Slides were stained in 5-10% Giemsa solution (pH = 6.8) for 10-12 min.

Nine series were analyzed: three series each of slow- and fast-growing Kandalaksha herring (Chupa) and slow-growing Onega herring (Kolezhma). One metaphase plate from each blastula was examined.

RESULTS

Adults

Three different karyotypes have been found in the White Sea herring: $2n = 50$, $2n = 51$, $2n = 52$; NF = 60. The karyotype $2n = 52$ (Fig. 2A) was the most common. This karyotype has the following features:

- chromosomal formula - 6m + 2sm + 44a; three pairs of metacentrics and one pair of submetacentrics may be identified by measurements of relative length (RL) and centromeric index (CI); among acrocentrics it is possible to identify three pairs of the smallest ones, the remaining chromosomes form a row gradually decreasing in length (Table II);
- the absolute length of the chromosomes varies from 1.5 to 6.0 µm;
- in some metaphase plates, of several specimens, 1-2 subtelocentric chromosomes were found;

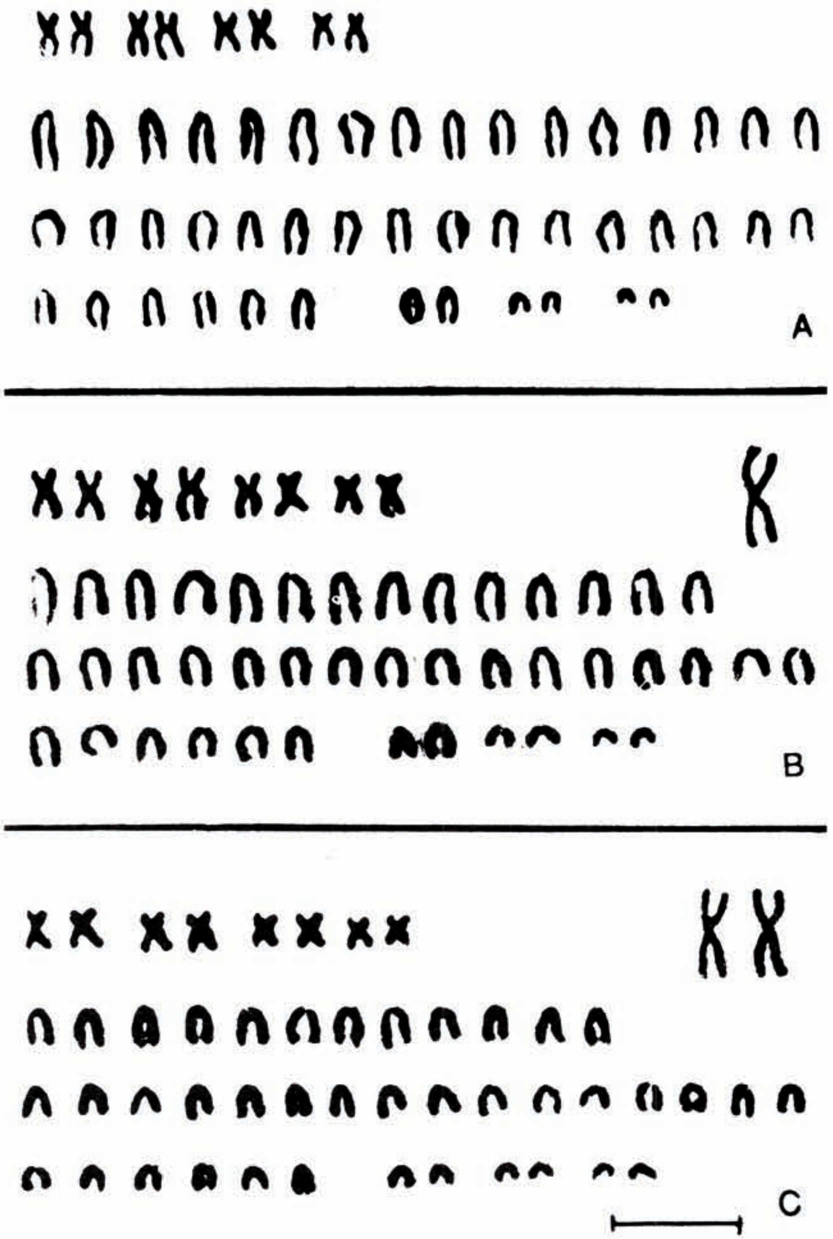

Fig. 2. - Karyotypes of the White Sea herring with 2n = 52, NF = 60 (A); 2n = 51, NF = 60(B); and 2n = 50, NF = 60(C). Scale = 10 µm.

- in the metaphase plates with low condensation of chromatin, satellite chromosomes and chromosomes with secondary constrictions were sometimes visible.

Karyotypes 2n = 51 and 2n = 50 contain, respectively, 1 or 2 large metacentrics more and 2 or 4 acrocentrics less than karyotype 2n = 52. These metacentrics are about twice as large as the large acrocentrics (Fig. 2B, C, Table II). Comparison of the karyotypes described above showed that each large metacentric in the karyotypes 2n = 51 and 2n = 50 is a result of fusion of two acrocentrics (Robertsonian fusion). No chromosomal differences between males and females have been found.

Karyotypes of the herring from different regions of Kandalaksha and Onega Bays, with the same diploid number, did not differ in the relative length or centromeric index of the chromosomes (Lajus, 1989a, Tables I-III).

In some locations, where the proportion of fishes with 2n = 51 and 2n = 50 is relatively high (Pushlakhta and Suzma), sometimes individuals with modal chromosome number 2n = 52 had some cells with 2n = 51.

Comparison of the different groups of White Sea herring from the same location, in the frequency of chromosomal rearrangement, indicates the lack of statistically significant differences between them (t-test for comparing of shares according to Lakin (1990) was used).

The following groups were compared: (1) collected in different years; (2) spawning and feeding herring; (3) slow- and fast-growing herring.

The absence of statistically significant differences allows all samples from the same location to be pooled for further analysis.

Larvae

In slides of larval material, the number of mitotic plates suitable for karyotyping was usually less than in adults or in the embryos at the stage of early eye-pigmentation. In all cases karyotypes 2n = 52, NF = 60 were found.

Embryos at the stage of early eye-pigmentation

Observations of the herring's spawning behavior indicated a wide dispersion of eggs and sperm in the spawning grounds (Blaxter and Hunters, 1982; Lajus, 1989b). So, in small areas of a spawning ground there were offsprings of many parental fishes. There-

Table II. - Relative length (RL, %%) (M ± m, $p < 0.05$) and centromeric index (CI, %%) (M ± m, $p < 0.05$) of chromosomes in karyotypes of the White Sea herring with 2n = 52 (26 metaphase plates from 26 slow-growing herrings (Chupa/ Kandalaksha Bay) were examined) and with 2n = 50 (10 metaphase plates from 2 herrings (Pushlakhta/Onega Bay) were examined).

N° of chromosomal pairs	2n = 52		N° of chromosomal pairs	2n = 50		Type
	RL	CI		RL	CI	
-	-	-	1	9.98 ± 0.12	49.0 ± 0.4	m
1	4.47 ± 0.03	47.2 ± 0.7	2	4.52 ± 0.10	47.1 ± 0.9	m
2	4.38 ± 0.02	40.0 ± 0.5	3	4.33 ± 0.07	39.2 ± 0.5	m
3	3.58 ± 0.05	44.3 ± 0.7	4	3.60 ± 0.07	43.8 ± 1.0	m
4	3.37 ± 0.05	38.2 ± 0.8	5	3.47 ± 0.11	39.8 ± 1.0	sm
5 - 23	from 5.32 to 3.24	-	6 - 22	from 5.32 to 3.25	-	a
24	2.76 ± 0.04	-	23	2.71 ± 0.08	-	a
25	1.94 ± 0.03	-	24	1.91 ± 0.08	-	a
26	1.62 ± 0.05	-	25	1.52 ± 0.12	-	a

Table III. - Number of secondary constrictions and satellite chromosomes per cell in blastula embryos.

Date of sampling	Number of parents and their diploid number	Number of the embryos at the stage of the early eye-pigmentation observed				Number of blastula cells	Number of cells with number of secondary constrictions							Number of cells with number of satellite chromosomes					
		Total	2n = 52	2n = 51	2n = 50	observed	0	1	2	3	4	5	Mean, M ± m	0	1	2	3	4	Mean, M ± m
Chupa/Kandalaksha Bay, slow-growing																			
Apr 1986	5, 2n = 52 in all	30	30	0	0	20	0	3	6	6	5	0	2.65 ± 0.233	0	3	8	4	5	2.55 ± 0.235
Apr 1986	2, 2n = 52 in all	30	30	0	0	23	1	2	7	9	4	0	2.56 ± 0.216	0	4	7	5	7	2.65 ± 0.232
Apr 1987	2, 2n = 52 in all	30	30	0	0	17	1	2	4	4	5	1	2.76 ± 0.327	1	2	4	4	6	2.71 ± 0.306
Total	11, 2n = 52 in all	90	90	0	0	60	2	7	17	19	14	1	2.65 ± 0.144	1	11	29	11	18	2.63 ± 0.145
Chupa/Kandalaksha Bay, fast-growing																			
Jun 1986	2, 2n = 52 in all	30	30	0	0	11	0	2	2	2	4	1	3.00 ± 0.405	1	0	3	3	4	2.82 ± 0.377
Jun 1986	3, 2n = 52 in all	30	30	0	0	20	0	1	4	10	5	0	2.95 ± 0.184	1	1	5	7	6	2.80 ± 0.247
Jun 1986	4, 2n = 52 in all	30	30	0	0	18	1	3	3	5	6	0	2.67 ± 0.302	0	1	6	5	6	2.88 ± 0.227
Total	9, 2n = 52 in all	90	90	0	0	49	1	6	9	17	15	1	2.86 ± 0.160	2	2	14	15	16	2.84 ± 0.152
Kolezma/Onega Bay, slow-growing																			
Total	5, 2n = 52 in all	30	30	0	0	23	0	4	7	7	5	0	2.57 ± 0.216	1	2	7	8	5	2.61 ± 0.224
Total	4, 2n = 52 in all	30	29	1	0	20	0	2	4	8	6	0	2.90 ± 0.216	0	0	7	7	6	2.95 ± 0.134
Total	6, 2n = 52 in all	30	30	0	0	19	0	3	2	10	3	1	2.84 ± 0.245	1	0	5	8	5	2.84 ± 0.232
Total	15, 2n = 52 in all	90	89	1	0	62	0	9	13	25	14	1	2.76 ± 0.129	2	2	19	20	16	2.79 ± 0.124

fore the sample of eggs from a small part of a spawning ground may be a sample representative of the whole population.

Karyotypes of the White Sea herring are differentiated by the presence or absence of one or two well-visible large metacentric chromosomes (Lajus, 1989a). This allows one metaphase plate only to be analyzed for the identification of each individual karyotype.

Pooled samples from Kandalaksha and Onega Bays differed significantly ($p < 0.001$) from each other in the frequency of chromosomal rearrangement (Table I).

Blastula embryos

A large part of the previous chromosomal data on the White Sea herring was obtained using blastula embryos (Skvortzova, 1975). My studies (Lajus, 1987, 1990) did not support the data of Skvortzova on differences between the White Sea herring populations in the number of chromosomes. However, Skvortzova also found differences in the number of satellite chromosomes and chromosomes with secondary constrictions. Therefore this part of the article is devoted to analysis of these structures.

Metaphase plates from blastula embryos do not allow the precise number of chromosomes and chromosomal arms to be estimated. Due to the overlapping of chromosomes it is impossible to distinguish a chromosome with secondary constriction from two chromosomes lying near one another. So I used series in which the karyotype was analyzed on parental fishes and embryos at the stage of early eye-pigmentation (Table III).

The number of chromosomes with secondary constrictions per cell varied from 0 to 5, the average for the sample ranged from 2.56 to 3.00 (Table III, see also fig. 4 in Lajus (1989a)).

Satellite chromosomes were analyzed in the same cells. They were usually associated with acrocentrics of large or middle size. The number of satellite chromosomes per cell varied from 0 to 4, the average for the sample was from 2.55 to 2.95 (Table III, see also fig. 4 in Lajus (1989a)). Both the number of the satellite chromosomes, and of the

Table IV. - Frequency of chromosomal rearrangement of the White Sea herring. 1) Only adults; 2) only embryos at the stage of early eye-pigmentation; 3) among them: 228 adults, 143 larvae, 759 embryos at the stage of early eye-pigmentation; 4) among them: 113 adults, 713 embryos at the stage of early eye-pigmentation ; 5) NS: not significant ($p > 0.05$), *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

N	Location of sampling	Number of specimens	Frequency of rearrangement	Statistical significance of differences 5)									
				1	2	3	4	5	6	7	8	9	10
1	Chavanga	61 1)	0.000	#									
2	Pila	29 1)	0.000	NS	#								
3	Kasian	321 2)	0.002	NS	NS	#							
4	Chupa	1130 3)	0.003	NS	NS	NS	#						
5	Pongoma	32 1)	0.000	NS	NS	NS	NS	#					
6	Sorokskaya	66 1)	0.076	**	*	***	***	*	#				
7	Kolezhma	826 4)	0.043	**	NS	***	***	NS	NS	#			
8	Kiy	71 1)	0.078	***	*	***	***	*	NS	NS	#		
9	Pushlakhta	97 1)	0.103	***	**	***	***	**	NS	**	NS	#	
10	Suzma	45 1)	0.133	***	**	***	***	***	NS	**	NS	NS	#

chromosomes with secondary constrictions, varied greatly in different cells of one sample. No differences between herring stocks were found (Table III).

Data obtained indicate that differences between samples in the frequency of chromosomal rearrangement are due to geographic location of sampling only. All differences between samples collected in the same location may be considered as occasional ones. No differences in the number of satellite chromosomes or chromosomes with secondary constrictions have been found.

To show the overall picture of geographic variation of chromosomal complements of the White Sea herring, all samples from the same location for adults, larvae and embryos on the stage of early eye-pigmentation were pooled (Table IV).

The frequency of the rearrangement increases from north-west to south-east of the White Sea. The area of herring with a low frequency of the rearrangement (0.000 - 0.005) includes Kandalaksha Bay and adjacent parts of the Basin. In the interior parts of Onega Bay the herring have a higher frequency of the rearrangement (0.04-0.08). The herring from Dvina Bay have the highest frequency found (0.133). In the border between Onega and Dvina Bays locations (Pushlakhta) the frequency of the rearrangement is intermediate (0.103).

DISCUSSION

The main difficulty of the chromosomal analysis of fishes is the low number of dividing cells in adult fishes, especially in the spawning period. At the same time, the data obtained in this period are the most informative for population analysis of such mobile fishes as herring. This fact is probably a key for understanding why some previously reported karyological data of the White Sea herring are not confirmed by those presented here.

Skvortzova (1974a, 1975) reported that the spring-spawning slow-growing herring of Kandalaksha Bay (Chupa) have 2n = 52, while summer-spawning fast-growing herring of the same location and slow-growing herring from Onega (Kiy) and Dvina Bays (Yagri) have 2n = 54. These groups differ in number of the secondary constrictions (0 and 2-4 respectively) and the satellite chromosomes (0 and 2-4). In order to obtain a sufficient number of metaphases for analysis, the blastula squash technique was used in these studies. This blastula technique was widely used earlier, but due to difficulties of chromosome counting and analysis of their morphology it is not suitable for accurate karyotyping in many fish species (Ohno *et al.*, 1965; Roberts, 1967). Subsequent studies on adult herring and embryos revealed that the blastula technique does not yield adequate results in this species also (Lajus, 1987, 1989a; Klinkhardt, 1993b). Also in support of this view is the discrepancy of data on Atlantic herring blastulae (2n = 54), presented by Skvortzova (1974b), compared with those obtained after colchicine pretreatment (Roberts, 1966; Lajus, 1992, 1994; Klinkhardt, 1993a) (2n = 52). In a case when Skvortzova (1975) used colchicine pretreatment (in larvae) the results were quite similar to data from other studies. Data on the number of secondary constrictions and satellite chromosomes from blastulae, presented here (Table III) also provide evidence for the absence of differences between different populations in the number of these structures.

Krysanov (1978) used a squash technique with colchicine pretreatment in spawning herring. He found intrapopulation differences in the chromosome number of the spawning herring from Onega Bay (Kolezhma) (16 fishes had 2n = 52, NF = 58 and 2 fishes had 2n = 54, NF = 58). Krysanov explained these differences by Robertsonian rearrangement, however he did not explain the absence of fishes with 2n = 53 in spawning grounds where fishes with 2n = 52 and 2n = 54 spawn together. I examined 113 adults and 713 embryos from Kolezhma, but did not find herring with 2n = 54 and/or with NF = 58. Unfortunately, direct detailed comparison of karyotypes is impossible, because illustrations are not given in Krysanov's paper.

Therefore, all previous findings of differences between the White Sea herring populations were not supported by my results. According to the data obtained, the only chromosomal difference between the White Sea herring populations which is known at present is due to Robertsonian polymorphism 2n = 52, 51, 50.

The frequency of this rearrangement is rather low in all the White Sea herring stocks. So it is necessary to use a large number of specimens to obtain statistically significant results. Therefore a special modification of the chromosome technique, for embryos, has been developed. The advantages of this technique are as follows:

- the possibility of analyzing a large number of specimens;
- the material may be sampled without special tools: eggs of the White Sea herring are usually laid on littoral or sublittoral vegetation to a depth of 5 m, live eggs are often cast ashore and may be collected;
- easy manipulation with embryos: transportation and additional incubation (if necessary) of eggs, and making the slides, are possible in the field.

Although this modification may be used during spawning the period only, it seems to be promising for the White Sea herring chromosomal investigations.

The good agreement of data presented here with those reported recently by Klinkhardt (1993a, b) should be noted. Klinkhardt studied 21 slow-growing and 18 fast-growing herring from Kandalaksha Bay. All specimens had 2n = 52 and configuration 6m + 2st + 44a, NF = 58, without any polymorphism. The fourth chromosome pair was classified as subtelocentric, whereas I classified it as submetacentric according to centromeric index (38-40%, Table II). Probably Klinkhardt used, for analysis metaphase plates with more condensed chromatin and, consequently, obtained a smaller value of arm ratio of bi-armed chromosomes. As in my observations (Lajus, 1989a), Klinkhardt noted heteromorphism of large acrocentric chromosomes. Silver staining revealed that this heteromorphism is not due to the presence of a nucleolar organizer region, which is located between the 4th and 6th acrocentrics (Klinkhardt, 1993a, fig. 9a). It may be associated with one of the pairs of satellite chromosomes, which are well visible in blastula metaphase plates (Lajus, 1989a).

Most of the Clupeidae have diploid chromosome number, 2n = 44-54 and a number of chromosomal arms, NF = 46-60 (Doucette and Fitzsimons, 1988). Their karyotypes are close to the ancestral type of Teleostei, presumably containing 48 acrocentrics (Ohno, 1970). This is evidence of a low rate of chromosomal evolution of Clupeidae, in comparison with other fishes. Chromosomal polymorphism, described here, is found in Clupeidae for the first time. It can be noted, that 6 or 7 of 15 investigated fish species from the White Sea (excluding *Clupea pallasi*) are not chromosomally monomorphic: *Myoxocephalus scorpius* (Vasil'ev, 1985; Lajus, 1992); *Salmo salar* (Semenova, 1987); *Coregonus lavaretus* (Yershov and Lajus, 1993); *Gymnacanthus tricuspis, Eleginus navaga, Lumpenus fabricii* and, probably, *Zoarces viviparus* (Lajus, 1992).

When chromosomal polymorphism is investigated, it is usually difficult to determine what karyotype is ancestral. In the White Sea herring the analysis of the related forms permits affirmation that the ancestral karyotype is 2n = 52 and, consequently, a fusion of acrocentrics with reduction of the chromosomal number takes place. The 52-chromosome complement is typical both for *C. pallasi pallasi* (Ohno *et al.*, 1969) and for *C. harengus* (Roberts, 1966; Lajus, 1992, 1994; Klinkhardt, 1993a). The number of subtelocentric chromosomes in the karyotype of the Atlantic herring is, probably, somewhat larger than in the Pacific one (chromosomal formula is 6m + 2sm + 2st + 42a (Lajus, 1994). So the karyotype 6m + 2sm + 44a seems to be ancestral for *C. harengus* also.

It is considered that divergence of the White Sea populations began about 10 thousand years ago (Deriugin, 1928; Berg, 1934; Andriashev, 1957). Division of frequency of the rearrangement (0.13 for Dvina Bay herring) by the time of rearrangement yields a rate of fixation of the chromosomal rearrangement: 1.3×10^{-5} rearrangements per year. The mean rate of fixation in Teleostei may be assessed by means of division of the average number of rearrangements per year per species (about 2.9×10^{-8}, Bush *et al.*, 1977) by the proportion of polymorphic species (0.01, Vasil'ev, 1985). The result (2.9×10^{-6}) shows, that in the White Sea herring (population of Dvina Bay) the rate of fixation is about 5 times higher than the average in Teleostei.

Fixation of the chromosomal rearrangement in a population depends on several factors and their combinations: genetic drive, inbreeding, meiotic drive and selection (White, 1978; Hedrick, 1981). Observations on herring spawning behavior indicate the high mixing of fishes in their populations. Populations are usually large, and individuals have a high mobility (Blaxter and Hunter, 1982). This decreases the role of the first two factors in explaining the fixation of a new chromosome morph and, conversely, increases the role of the two other factors.

Meiotic drive, even if rather slow, may have serious populational and evolutional consequences (Hedrick, 1981; Walsch, 1982). The advantage of meiotic drive as an explanation of fixation of chromosomal rearrangement is its ability to increase the rate of fixation of chromosomal rearrangement not only in small demes, but in large populations also (theoretical analysis by Walsch, 1982). Meiotic drive probably is a potentially important factor for chromosomal speciation, but its actual role is not quite clear at present (Gileva, 1990).

Selective advantage of individuals with reduced chromosome number may be due to new chromosomes, which contain a complex of associated genes (Sheel, 1972; Birshtein, 1987; Kirpichnikov, 1987). Moreover, an important factor of increasing of the frequency of chromosomal rearrangement, especially at low frequency, may be the enhanced fitness of heterozygotes, observed in some animals (Baker *et al.*, 1983; Christensen and Pedersen, 1982). After occupation of the White Sea, herring colonised a new environment, which resulted in chromosome alteration in the south-east part of the area. Rearrangement 2n = 52, 51, 50 was supported by selection and transmitted to all areas. Observed differences in frequency of rearrangement may be explained by the selection against herring with 2n = 51 and/or 2n = 50 in the north-west of the area or by presence of ecological barriers between stocks, which prevent gene flow.

The data obtained allow the following conclusions concerning population structure and evolution of the White Sea herring (Lajus, in press):

- all stocks of the White Sea herring have derived from the Pacific herring but not from the Atlantic and the Pacific herring as some authors considered (Dushkina, 1975; Skvortzova, 1975);

- the White Sea herring is not a single population. The viewpoint of Lapin and his supporters (e.g., Lapin, 1966; Lapin and Pokhiliuk, 1993) is incompatible with differences in the frequency of chromosomal rearrangement in herring from different localities. Differentiation of the White Sea herring stocks was also supported by recent studies of models based on fishery statistics (Naymark and Frolov, 1993). At the same time, the presence of Robertsonian polymorphism in all populations studied reveals the limited gene flow between them;

- chromosomal data allow identification of differences between stocks which inhabit different parts of the sea, but not between slow-and fast-growing herring. Slow- and fast-growing herring from the same location are closer to each other than slow- (or fast-) growing ones from different locations. Formerly it was considered (e.g., Rabinerson, 1925; Averintsev, 1927) that there are two large groups of the White Sea herring: fast-growing (*C. harengus pallasi marisalbi* var. a (Rabinerson)) and slow-growing (*Clupea harengus pallasi marisalbi* var. b (Rabinerson)), each forming stocks in different parts of the sea.

Therefore chromosome studies contribute to the general picture of the White Sea herring population structure. However, this method has the following limitations: populations can be compared in one parameter only - frequency of the chromosomal rearrangement. It is entirely possible, that evolution of some stocks (for instance, stocks from Kandalaksha Bay and adjacent parts of the Basin) did not involve with the divergence of chromosomal sets. Moreover, no chromosomal differences between populations from the same location were found. These populations, probably, have not diverged sufficiently to have detectable chromosomal differences.

As regards future population studies of herring of the North of Russia, I think that the chromosomal method could yield interesting results by comparing populations from distinct parts of the White Sea and adjacent parts of the area of Pacific herring: Mezen Bay, Chesha Bay, Pechora Bay and the Kara Sea. Concerning the populations already studied, other methods of population genetics such as protein electrophoresis and nucleic acid technologies seem to be informative. These methods are widely used in herring population studies (Anderson *et al.*, 1981; Kornfield *et al.*, 1982; Grant, 1984; Grant and Utter, 1984; Ryman *et al.*, 1984; Kornfield and Bogdanowicz, 1987; Schweigert and Withler, 1990). New opportunities are opened up also by the assessment of developmental stability and phenotypic variability. Use of these approaches may reveal possible reasons for the divergence between White Sea herring populations (Lajus, 1996, in press).

Acknowledgements. - I thank Oleg Ivanchenko for his help in all stages of my study, Julia Lajus, Inna Sokolova, and Olga Saranchova for help in preparation of the manuscript. This work was supported by the International Science Foundation and Russian Academy of Natural Sciences.

REFERENCES

ANDERSON L., RYMAN N., ROSENBERG R. & G. STAHL, 1981. - Genetic variability in Atlantic herring (*Clupea harengus harengus*): Description of protein loci and population data. *Hereditas*, 95: 69-78.

ANDRIASHEV A.P., 1957. - Nekotorye voprosy amfiboreal'nogo rasprostraneniia s zamechaniiami o vozmozhnom proiskhozhdenii navagi i malopozvonkovoi sel'di [Same questions of amphiboreal distribution with notes on possible origin of navaga and low-vertebrate herring]. *In:* Materialy po kompleksnomu izucheniiu Belogo moria - Moskow-Leningrad, Izd. AN SSSR, 1: 117-125.

AVERINTSEV S.V., 1927. - Sel'di Belogo moria. Chast' 1 [Herring of the White Sea. Part I]. *Tr. NIRKh*, 3-4: 39-78.

AVERINTSEV S.V., 1928. - Sel'di Belogo moria. Chasti II i III [Herring of the White Sea. Parts II and III]. *Tr. NIRKh*, 3-4: 71-112.

BAKER R.J., KOOP B.F. & M.W. HAIDUK, 1983. - Resolving systematic relationships with G-bands: a study of five genera of South American cricetine rodents. *Syst. Zool.*, 32(4): 403-416.

BERG L.S., 1934. - Ob amfiboreal'nom (preryvistom) rasprostranenii morskoi fauny v severnom polusharii [On amphiboreal (discontinuous) distribution of sea fauna in North hemisphere]. *Izv. Geogr. obtsch.*, 66(1): 69-78.

BIRSHTEIN V.J., 1987. - Tsytogeneticheskie i molekuliarnye aspekty evoliutsii pozvonochnykh [Cytogenetic and molecular aspects of vertebrate evolution]. 284 p. Moscow. Nauka Press.

BLAXTER J.H.S. & H. HUNTERS, 1982. - The biology of the clupeoid fishes. *Adv. Mar. Biol., Lond.*, 20: 1-223.

BUSH G.L., CASE S.W., WILSON A.C. & J.L. PATTON, 1977. - Rapid speciation and chromosomal evolution in mammals. *Proc. Nat. Acad. Sci. USA*, 74(9): 3942-3946.

CHRISTENSEN K. & H. PEDERSEN, 1982. - Variation in chromosome number in the blue fox (*Alopex lagopus*) and its effect on fertility. *Hereditas*, 97(2): 211-215.

DENTON T.E., 1973. - Fish Chromosome Methodology. 166 p. Springfield, Illinois: C.C. Thomas.

DERIUGIN K.M., 1928. - Fauna Belogo moria i usloviia ee suschestvovaniia [Fauna of the White Sea and conditions of its existence]. 455 p. Issledovaniia morei SSSR 7/8, Leningrad, Hydrological Institute Press.

DMITRIEV N.A., 1946. - Biologia I promysel seldi v Belom more [Biology and fishery of herring in the White Sea]. 88 p. Pitshepromizdat Press, Moscow.

DOUCETTE A.J. & J.M. FITZSIMONS, 1988. - Karyology of Elopiform and Clupeiform fishes. *Copeia*, 1988(1): 124-130.

DUSHKINA L.A., 1975. - Pigmentatsiia morskikh sel'dei roda Clupea kak odin iz vozmozhnykh pokazatelei ikh proiskhozhdeniia [Pigmentation of herring of genera *Clupea* as a one of possible indicators of their origin]. *In:* Issledovaniia Fauny Morei, Leningrad, Nauka Press, 16: 227-254.

DUSHKINA L.A., 1988. - Biologiia morskikh sel'dei v rannem ontogeneze [Biology of marine herring in early onthogenesis]. 216 p. Moscow, Nauka Press.

GILEVA E.A., 1990. - Khromosomnaia izmenchivost' i evoliutsiia [Chromosomal variation and evolution]. 144 p. Moscow. Nauka Press.

GOLD J.R.A., 1974. - A fast and easy method for chromosome karyotyping in adult teleosts. *Progr. Fish-Cult.*, 36(3): 169- 171.

GRANT W.S., 1984. - Biochemical population genetics of Atlantic herring, *Clupea harengus*. *Copeia* (2): 357-364.

GRANT W.S. & F.M. UTTER, 1984. - Biochemical population genetics of Pacific herring (*Clupea pallasi*). *Can. J. Fish. Aquat. Sc.*, 41: 856-864.

HEDRICK G.H., 1981. - The establishment of chromosomal variants. *Evolution*, 35(2): 322-332.

KIRPICHNIKOV V.S., 1987. - Genetika i selektsiia ryb [Genetics and Selection of Fishes]. 520 p. Leningrad. Nauka Press.

KLINKHARDT M.B., 1993a. - Cytogenetics of the herring (*Clupea harengus* L.) 1. Karyotypes of North European stocks. *Cytobios*, 75: 113-128.

KLINKHARDT M.B., 1993b. - Cytogenetics of the herring (*Clupea harengus* L.) 2. Karyotypes of White Sea herring groups. *Cytobios*, 75: 149-156.

KORNFIELD I. & S.M. BOGDANOWICZ, 1987. - Differentiation of mitochondrial DNA in Atlantic herring, *Clupea harengus*. *Fish. Bull.*, 85: 561-568.

KORNFIELD I., SIDELL B.D. & P.C. GAGNON, 1982. - Stock definition in Atlantic herring (*Clupea harengus harengus*): genetic evidence for discrete fall and spring spawning populations. *Can.. J. Fish. Aquat. Sc.*, 39: 1610-1621.

KRYSANOV E.Y., 1978. - Ob izmenchivosti chisla khromosom u sel'di [On the variability of chromosome numbers in herring], pp. 94-98. *In:* Ekologia ryb Belogo morya. Moscow. Nauka press.

LAJUS D.L., 1987. - Novye dannye o kariologicheskikh osobennostiakh sel'dei, nerestiaschikhsia v Kandalakshskom zalive Belogo moria [The new data on karyological peculiarities of herring spawning in the Kandalaksha Bay (White Sea)]. *Dokl. AN SSSR,* 297(1): 254-256.

LAJUS D.L., 1989a. - Preobrazovaniia khromosomnykh naborov belomorskoi sel'di (*Clupea pallasi marisalbi* (Berg) (Teleostei, Clupeidae) [Chromosomal transformations in the White Sea herring (*Clupea pallasi marisalbi* (Berg) (Teleostei, Clupeidae)]. *Trudy Zool. Inst.,* 192: 113-125.

LAJUS D.L., 1989b. - Osobennosti ekologii i populiatsionnoi struktury sel'di Belogo moria [Ecology and population structure of the herring of the White Sea]. Ph.D thesis, 202 p. Zooligical Institut, USSR Academy of Sciences. Leningrad.

LAJUS D.L., 1990. - Populiatsionnaia struktura belomorskoi sel'di: dannye kariologicheskogo analiza [Population structure of White Sea herring: karyological data]. *Trudy Zool Inst.,* 227: 4-16.

LAJUS D.L., 1991. - Analiz fluktuiruiuschei asimmetrii kak metod populiatsionnykh issledovanii belomorskoi sel'di [Analysis of fluctuating asymmetry as a method of population studies of the White Sea herring]. *Trudy Zool. Inst.* 235: 113-122.

LAJUS D.L., 1992. - Khromosomnye nabory 13 vidov ryb Belogo moria [Chromosome sets of 13 species of fishes from the White Sea], pp. 179-180. *In:* Problemy izucheniia, ratsional'nogo ispol'zovaniia i okhrany prirodnykh resursov Belogo moria. Reg. konf. Tez. dokl. Petrozavodsk.

LAJUS D.L., 1994. - Opisanie khromosomnogo nabora mnogopozvonkovoi sel'di *Clupea harengus* L. iz Belogo moria [Description of chromosome set of high-vertebrae herring, *Clupea harengus* L. from the White Sea]. *Vopr. Ikht.,* 34(4): 572-574.

LAJUS D.L., 1996. - What is the White Sea herring: a new concept of the population structure. *In:* Proc. 8th Congr. Soc. Europ. Ichtiol. *Boel. Inst. Nac. Oceanogr.,* spec. issue (in press).

LAKIN G.F., 1990. - Biometry. 352 p. Moscow. Vysshaya shkola.

LAPIN J.E., 1966. - Sel'di Belogo moria kak biologicheskoe tseloe [Herring of the White Sea as an biological whole], pp. 5-28. *In:* Zakonomernosti dinamiki chislennosti ryb Belogo moria i ego basseina. Moscow. Nauka Press.

LAPIN J.E. & V.V. POKHILIUK, 1993. - O prostranstvennoi dinamike pokolenii belomorskoi sel'di, *Clupea pallasi marisalbi* [On the spatial dynamics of generations in the White Sea herring, *Clupea pallasi marisalbi*]. *Vopr. Ikht.,* 33(3): 367-371.

McPHAIL J.D. & R.L.A. JONES, 1966. - A simple technique for obtaining chromosomes from teleosts fishes. *J. Fish. Res. Bd. Can.,* 23(5): 767-768.

NAYMARK E.B. & S.V. FROLOV, 1993. - Modelirovanie populiatsii belomorskoi sel'di *Clupea pallasi marisalbi* [Modeling of the White Sea herring, *Clupea pallasi marisalbi*, populations]. *Vopr. Ikht.,* 33(3): 359-366.

OHNO S., 1970. - Evolution by Gene Duplication. 160 p. Berlin etc..

OHNO S., MURAMOTO J., KLEIN J. & N. ATKIN, 1969. - Diploid-tetraploid relationships in Clupeoid and Salmonoid Fish. *In:* Chromosomes Today, II, Oxford Chromosome Conference, 2: 139-148.

PROKOFIEVA A.A., 1935. - Morfologiia khromozom nekotorykh ryb i amfibii [Morphology of chromosomes of some fishes and amphibias]. *Trudy Inst. Genet.,* 10: 153-178.

RABINERSON A.I., 1925. - Materialy po issledovaniiu belomorskikh sel'dei [Contribution to the natural history of the White Sea herring]. *Trudy Inst. Izuch. Severa,* 25: 146 p.

RASS T.S., 1985. - O sistematike morskikh sel'dei roda Clupea. [On the systematic of marine herring of Genus *Clupea*]. *Trudy Zool. Inst.,* 179: 53-56.

RASS T. & A. WHEELER, 1991. - The nomenclature of the Pacific herring, *Clupea pallasii* Valenciennes, 1847. *J. Fish Biol.,* 39: 137-138.

ROBERTS F.L., 1966. - Cell culture of fibroblasts from *Clupea harengus* gonads. *Nature,* 212(5070): 1592-1593.

ROBERTS F.L., 1967. - Chromosome cytology of the Osteichthyes. *Progr. Fish-Cult.*, 29(2): 75-83.

RYMAN N., LAGERCRANTZ U., ANDERSON L., CHAKRABORTY R. & R. ROSENBERG, 1984. - Lack of correspondence between genetic and morphologic variability patterns in Atlantic herring (*Clupea harengus*). *Heredity*, 53: 687-704.

SCHWEIGERT J.F. & R.E. WITHLER, 1990. - Genetic differentiation of Pacific herring based on enzyme electrophoresis and mitochondrial DNA analysis. *In:* Amer. Fish. Soc. Symp., 7: 459-469.

SEMENOVA O.V., 1987. - Khromosomnaia izmenchivost' atlanticheskogo lososia iz estestvennykh populiatsii rek basseina Belogo moria [Chromosomal variability of Atlantic salmon from native populations of the White Sea basin rivers], pp. 173-182. *In:* Geneticheskie issledovaniia morskikh gidrobiontov. Moscow. Nauka Press.

SHEEL J.J., 1972. - Rivuline karyotypes and their evolution (*Rivuline*, Cyprinodontidae, Pisces). *Ztschr. Zool. Syst. Evolutionsforsch.*, 10(3): 180-229.

SHELENKOVA N.J., 1986. - Kariologiia nerki (*Oncorhynchus nerka* Walb.) dvukh izolirovannykh populiatsii na Kamchatke [Karyology of the sockey (*Oncorhynchus nerka* Walb.) of two isolated populations of Kamchatka]. *Tsytologia*, 28(7): 735-739.

SKVORTZOVA T.A., 1974a. - Kariologicheskie dannye, svidetel'stvuiuschie o neodnorodnosti belomorskoi sel'di (*Clupea harengus pallasi n. maris-albi* Berg) [Karyological evidences of heterogenity of the White Sea herring (*Clupea harengus pallasi n. maris-albi* Berg)], p. 26. *In:* Otchetnaia nauchnaia sessiia po itogam rabot za 1973 g. Zoologicheskogo instituta AN SSSR, Tez. dokl. Leningrad.

SKVORTZOVA T.A., 1974b. - Khromosomnye kompleksy okeanicheskoi sel'di [Chromosome complexes of herring], pp. 199-200. *In:* Biologiia promyslovykh ryb i bespozvonochnykh na rannikh stadiiakh razvitiia. Vses. konf. Tes. dokl. Murmansk:.

SKVORTZOVA T.A., 1975. - Khromosomnye kompleksy belomorskoi sel'di (*Clupea harengus pallasi n. maris-albi* Berg) i salaki (*Clupea harengus harengus n. membras*) [Chromosome complexes of the White Sea herring (*Clupea harengus pallasi n. maris-albi* Berg) and the Baltic herring (*Clupea harengus harengus n. membras* L.)]. *Issl. Fauny Morei*, 16: 104-108.

SVARDSON G., 1945.- Chromosome studies on *Salmonidae*. *In:* Reports from the Swedish State Institute Fresh-Water Fish Researches. 23 p. Stockholm.

VASIL'EV V.P., 1985. - Evoliutsionnaia kariologiia ryb [Evolutionary karyology of fish]. 304 p. Moscow, Nauka Press.

WALSCH J.B., 1982. - Rate of accumulation of reproductive isolation by chromosome rearrangements. *Amer. Nat.*, 120(4): 510-522.

WHITE M.J., 1978. - Models of Speciation, 6: 455 p. San Francisco: Freeman.

WHITEHEAD P.J.P., 1985. - Clupeoid fishes of the world (Suborder *Clupeoidei*). *In:* FAO Species Catalogue 7(125), P 1, 304 p. Rome.

YERSHOV P.N. & D.L LAJUS, 1993. - Khromosonyi nabor prokhodnogo siga *Coregonus lavaretus pidgian* iz reki Keret' basseina Belogo moria [Chromosomal variability of the anadromous whitefish *Coregonus lavaretus pidschian* in the Keret river (White Sea)]. *Tsytologia*, (11/12): 86-95.

Reçu le 07.02.1995.
Accepté pour publication le 16.02.1996.